UNITED STATES DEPARTMENT OF AGRICULTURE
BUREAU OF HOME ECONOMICS
Washington, D.C.

MEASURING THE VITAMINS 1/

By Hazel E. Munsell

The vitamins have been known for little more than two decades and yet the reports of studies on these important food factors run into the thousands. It might seem that there would be little left to learn about them but it is only within the last few years that we have had any definite knowledge concerning their chemical nature. From the available evidence it is apparent that chemically they are entirely unrelated and must be treated as individuals and not as members of a group having special characteristics in common.

At the present time we generally deal with six vitamins although evidence has been presented for the possible existence of several others. A diet made up of a variety of natural food products will ordinarily contain a supply of each adequate for the needs of the body. Oftentimes, however, peculiarity of food habits, economic conditions, or other reasons may bring about the use of a restricted diet that supplies only suboptimal amounts of one or more. Many instances of ill health, lowered resistance, to certain types of infections, and chronic disease are in all probability the result of long continued use of diets restricted to foods that do not furnish ample amounts of the vitamins. Wolff in Holland (1) and Moore in England (2) have recently presented evidence along this line. Wolff reports the results obtained in autopsy examinations of 957 human livers. In those cases where death was due to accident or acute disease the livers showed a consistently higher vitamin A content than in cases when death was caused by some chronic disease. Moore examined 300 specimens and found subnormal amounts of this vitamin in cases of organic heart disease, nontuberculous respiratory diseases, and in septicemias and certain septic conditions.

Another type of evidence showing the importance of including optium amounts of vitamins in the diet is that obtained by Ellis (3) and Whalen (4) working with Sherman at Columbia University. These investigators showed that when the vitamin G content of the diet of white rats is increased there follows a marked improvement in the nutritional performance of the animals. A higher average weight is attained at all ages. Increasing the vitamin G content of the mothers' diet results in higher average weights for the young at weaning. The actual number of young born are not increased but there are a larger per cent weaned. This last was interpreted as indicating that the requirement for vitamin G for lactation is greater than the requirement of this vitamin for growth. Whalen gives the ratio as about 2 to 1. An abundance of vitamin G causes early maturity but does not affect the duration of the breeding period

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1/ This article was published in The Medical and Professional Women's Journal, October 1933.

although on a low vitamin G intake the length of the breeding period is significantly reduced.

Such evidence is impressive and cannot fail to convince those interested in nutrition with the importance of including in the diet at all times adequate sources of each of the vitamins. It is just as essential that we know which foods can be relied upon for these food factors as it is that we know where to get our calcium, phosphorus, and iron. This is especially important at the present time when we are feeding large numbers of people at public expense. In keeping the amount of money spent for food at the lowest possible minimum there is danger of including in the diet too few of those foods needed to supply adequate amounts of the vitamins.

Occurrence of vitamins in natural food products and the effects of the various methods of food preparation on the vitamin content has become an important part of research in the field of nutrition. The relative importance as a source of vitamins of a fairly large number of foods is known. Some of this information has been obtained from studies that had as the main objective the analysis of foods for their vitamin content and some of it has been obtained vicariously from studies undertaken to determine the chemical nature of the vitamins and their effect on the animal organism. However, when it comes to reliable quantitative values the situation is somewhat different. Not only are there very few data available but the methods used for obtaining them as well as the terms used to express the actual values are crude and cumbersome.

It is only within the last few years that any of the vitamins have even been tentatively identified chemically and therefore most of the analyses of foods have been done by feeding tests with animals. One of the earliest attempts to give quantitative expression to values obtained in this way was that of Sherman, LaMer, and Campbell (5). These investigators showed that the degree of protection against scurvy afforded a guinea pig was directly proportional to the amount of vitamin C supplied in its diet. As a result of this work it is possible to express the vitamin C values of foods in terms of units, a unit being that amount which, when fed as a daily allowance, just suffices to completely protect a standard guinea pig from scurvy. This method of expressing vitamin C values has been used extensively by investigators in this country.

Eddy (6) describes a method based on the work of Höjer (7) in which the condition of the teeth of guinea pigs is used as the criterion for judging the presence or absence of vitamin C in the food under test. This method has the advantage of a much shorter feeding period. It has not been used to any great extent, however, since it involves a working knowledge of histological technic and equipment for preparing the material for examination. Values obtained by this method are about one-half those obtained by the method of Sherman, LaMer, and Campbell.

A standardized procedure for the determination of vitamin A reported in the tenth revision of the United States Pharmacopoeia was the first vitamin method to be officially adopted. As originally worked out this test gave fairly satisfactory results but as work on vitamin A progressed it became apparent that it left much to be desired. However, if precautions are taken in the rearing of the young to be used in the tests

and if sufficiently large numbers of animals are employed, results may be obtained that are reasonably satisfactory.

In this method the unit of vitamin A is defined as that amount which when fed daily just suffices to support a gain of 3 grams per week in a standard test animal (rat) during an experimental feeding period of 4 to 8 weeks. In making feeding tests it is seldom that a dose is chosen that actually gives this standard gain. It is usual to feed the test food at several different levels using a number of animals for each. After a few preliminary tests it is possible to calculate one dose that will give a gain slightly greater than 3 grams per week, one a gain somewhat less than 3 grams and a third approximately 3 grams per week. The average gains in weight are then plotted against the amount of test food and from the resulting curve it is possible to derive a figure for the amount of test food required to give a rate of gain of 3 grams per week. The results can be accepted with greater assurance of course if more than three points are determined. The reliability of the results may also be judged from the smoothness of the curve obtained. This method of deriving the unit value has been in use in the writer's laboratory for nearly ten years. It was recently elaborately described by Coward (8).

Many improvements have been introduced in the method as originally described. A recent review of vitamin A technic by Gudjonnson (9) stresses the importance of standardized feeding of the mothers and the use of a test period of 8 to 10 weeks duration. Coward (10) however, presents data showing that the "increase in accuracy obtainable by the prolongation of a vitamin A test beyond a period of 3 weeks is too slight to justify generally the extra expenditure of time and labor which would be involved." She also found that to get equal degrees of accuracy about twice as many males as females must be used in a test. (11). Bacharach (12) has produced evidence that some of the vitamin A free diets may be lacking in factors other than vitamin A.

As early as 1922 Drummond (13) suggested a color test for vitamin A. Work was done along this line by several investigators. In 1926, Carr and Price (14) studied reagents which they thought might find application in the detection of this vitamin and as a result of their work the antimony trichloride test was developed. This test is characterized by an intense blue color obtained when a substance containing vitamin A is treated with a solution of antimony trichloride in chloroform and is considered by some to be specific for vitamin A. However, it has been used chiefly with oils having very little natural pigmentation. As now perfected this method gives results that are fairly consistent with those obtained by the biological method. Values generally expressed in terms of "blue units" are obtained by matching with standard glasses in a Lovibond tintometer.

Norris and Church (15) have shown that "a correlation can be made between the colorimetric assay for vitamin A using antimony trichloride, if the color value is compared when it is a linear function of the cod liver oil, and the biological assay." For an 8-week test period an average value of 7.6 blue units was obtained as equivalent to 10 biological units. These investigators emphasize the fact that the growth-promoting factors measured as vitamin A of plant and animal source, are not identical. Any chemical test cannot be applicable to the growth-promoting substances from both sources. Pure carotene, although having growth-promoting properties, does not give the blue color with antimony trichloride reagent.

Some of the earliest work on the chemistry of vitamins was done in connection with vitamin B. The antineuritic properties of some foods and of the extracts made from them were known even before the vitamin hypothesis was propounded. Many attempts have been made to isolate this vitamin and several investigators claim to have prepared crystalline products possessing properties of vitamin B. The material obtained by Windaus (16) seems to be the nearest approach to pure vitamin B. Siedell (17) was the first to suggest the method of concentrating the vitamin by absorbing it on Fuller's earth (Lloyd's reagent). An enormous amount of work has been done in studying the effect of deprivation of vitamin B in both birds and animals. Since pigeons kept on a diet devoid of vitamin B develop polyneuritis quite uniformly they have been used extensively in determining the value of foods as sources of this factor. Comparisons are generally made by determining either the amount of the food under test needed to cure well defined symptoms of polyneuritis in a specified time, or the amounts that will prevent these symptoms when the birds are given a diet uniformly free from vitamin B.

Since vitamin B has growth promoting-properties methods based on feeding tests with animals have also been used in making quantitative determinations. Sherman (18) defined a unit of vitamin B as that amount which when fed as a daily allowance resulted in net maintenance of a standard rat over an eight-weeks' test period.

It has been necessary to discredit much of the early work on vitamin B since we now know that at least two and probably more factors were involved instead of a single one as at first supposed. From the results obtained by Chase (19) Sherman now recommends a rate of gain of 3 grams per week during the test period as a suitable level for making comparative measurements of vitamin B values. Upon this basis a unit of vitamin B then becomes such an amount as will induce this rate of gain.

It was at first thought that the vitamin B values obtained earlier might be reinterpreted but such an attempt proved to be impracticable. Many times in the growth tests vitamin G was without doubt the first limiting factor rather than vitamin B while in other cases it was not possible to tell exactly which factor was responsible for the growth. The results obtained in experiments in which pigeons were used are difficult of interpretation on the basis of animal growth methods. Consequently, there are few reliable data on the vitamin B content of foods.

Less work has been done in connection with vitamin G than any of the others with the possible exception of vitamin E. Following the plan outlined for vitamin A, however, Bourquin (20) has described a unit of vitamin G as that amount which when fed daily to a standard rat results in an average gain of 3 grams per week during the test period. At this level of gain the results obtained are sometimes quite irregular and the writer has found that a slightly higher rate of gain, about 30 grams in 8 weeks, gives more consistent results.

Although vitamin D was the first vitamin to be identified chemically no strictly chemical method of analysis for this factor has been proposed. The spectroscopic method appears promising but there is still doubt as to

the specificity and reliability of some of the evidence offered and the method has never been generally accepted. The first method of analysis was the line test proposed by McCollum (21). Steenbock and Black (22) improved the basal diet described by McCollum and the one suggested by them is now quite generally used in vitamin D work. They defined a unit of vitamin D as the total amount of vitamin D which will produce a narrow line of calcium deposit in the rachitic metaphyses of the distal ends of the radii and ulnae of standard rachitic rats in a period of 10 days. This unit was accepted by the Council on Pharmacy and Chemistry of the American Medical Association for new and non-official remedies. It is also the unit used by the Wisconsin Alumnae Research Foundation and is generally employed by those preparing products made under the patent held by this foundation.

A unit similar to the one proposed by Steenbock and Black was set up by the Vitamin Assay Committee of the American Drug Manufacturers Association (23). Their unit is defined as the minimum average daily amount of vitamin D required to produce in 60 percent of the animals in any one group a degree of calcification represented by a narrow continuous line across the metaphysis of the leg bones of the rats which have been kept and fed under the conditions as specified in the assay. One A.D.M.A. unit is therefore equivalent to one-tenth of a Steenbock unit or values expressed in A.D.M.A. units will therefore be ten times the values given in Steenbock units.

Bills, Honeywell, Wirick and Nussmeier (24) describe a cod liver oil to be used as a standard of reference and give a method for accurately comparing an unknown with this standard. If the material tested is stated to have a cod liver oil coefficient of ten then it has a vitamin D value ten times that of the standard oil. An average cod liver oil is defined as that which induces 2 plus healing when administered as one fourth per cent of Diet 3143 (McCollum's) for five days to rats in which rickets has been induced. These investigators claim that a mixture of almost any four samples of oil may be used as an average sample. The method described by Bills for interpreting values obtained has not been extensively used although it has much to recommend it.

Viosterol, the trade name for irradiated ergosterol, dissolved in an inert oil, is generally standardized in terms of a standard cod liver oil. Thus visterol 20 D means a product having a vitamin D potency 20 times that of the standard cod liver oil. This standard oil is such that each 75 mg. contains one Steenbock unit of vitamin D. Each gram of oil, therefore, contains 13.33 Steenbock units.

Many other methods have been proposed for giving qualitative expression to vitamin values. The ones given below are those that have been used most frequently. It is plain to see that a universal system for expressing vitamin values is needed. A step toward the accomplishment of such an end has been taken by the Health Organization of the League of Nations. The Permanent Commission on Biological Standardization

appointed by this organization met in London in June 1931.2/ The conference on standardization of vitamins consisted of members from England, Sweden, Denmark, Holland, France, Germany, and the United States. As a result of this conference international standards of reference for four of the vitamins were proposed and have been accepted by the Permanent Commission on Biological Standards (25). These are as follows:

Vitamin A - The Conference recommends that carotene be accepted as an international provisional standard of reference for vitamin A and that a selected sample of cod liver oil be held in view as a possible secondary standard.

The unit of vitamin A recommended for adoption is the vitamin A activity of gamma (0.001 mg.) of the international standard.

Vitamin D - The Conference recommends that the standard solution of irradiated ergosterol at present issued from the National Institute of Medical Research, London, be adopted as international vitamin D standard for the next two years.

The unit of vitamin D recommended for adoption is defined as the vitamin D activity of 1 mg. of the international standard solution of irradiated ergosterol.

Vitamin B - The Conference recommends the adoption, as international standard, of the adsorption product of the antineuritic vitamin B prepared in the Medical Laboratory, Batavia (Java) by the method of Seidell, as described by Jansen and Donath.

The unit recommended for adoption is the antineuritic activity of 10 mg. of the international standard adsorption product.

Vitamin C - The Conference recommends the adoption as international standard of the fresh juice of the lemon, Citrus limonum.

The unit of the antiscorbutic vitamin C recommended for adoption is the vitamin C activity of 0.1 cc. of fresh juice of the lemon, Citrus limonum.

The conference made definite recommendations for the preparation and distribution of these standards. The International units for vitamins A and D have been adopted by the United States Pharmacopoeia Committee of Revision (26), and the Pharmacopoeial Vitamin Advisory Board has been organized for the preparation and distribution of vitamin standards within the United States. Through the generosity of the Health Organization of the League of Nations a limited supply of these standards for scientific research are now available and may be obtained by application to the Bureau

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2/ Since this article was written a second conference has been held in London at which beta-carotene was adopted as the standard for vitamin A and ascorbic acid for vitamin C.

of Chemistry and Soils, of the United States Department of Agriculture, or the United States Pharmacopoeial Vitamin Advisory Board, 43rd Street and Woodland Avenue, Philadelphia, Pennsylvania. A reference cod liver oil is now being prepared for distribution to manufacturers of "products having vitamin A and D potency."

The acceptance of the International Vitamin Standards should help to clarify much of the confusion that now exists in regard to vitamin values. If it is found possible to establish factors for the conversion of these older units much of the data already available can be interpreted in terms of International units.

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